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Clinical, polysomnographic and genome-wide association analyses of narcolepsy with cataplexy: a European Narcolepsy Network study

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SUMMARY

The aim of this study was to describe the clinical and PSG characteristics of narcolepsy with cataplexy and their genetic predisposition by using the retrospective patient database of the European Narcolepsy Network (EU-NN). We have analysed retrospective data of 1099 patients with narcolepsy diagnosed according to International Classification of Sleep Disorders-2. Demographic and clinical characteristics, polysomnography and multiple sleep latency test data, hypocretin-1 levels, and genome-wide genotypes were available. We found a significantly lower age at sleepiness onset (men versus women: 23.74 ± 12.43 versus 21.49 ± 11.83, $P = 0.003$) and longer diagnostic delay in women (men versus women: 13.82 ± 13.79 versus 15.62 ± 14.94, $P = 0.044$). The mean diagnostic delay was 14.63 ± 14.31 years, and longer delay was associated with higher body mass index. The best predictors of short diagnostic delay were young age at diagnosis, cataplexy as the first symptom and higher frequency of cataplexy attacks. The mean multiple sleep latency negatively correlated with Epworth Sleepiness Scale (ESS) and with the number of sleep-onset rapid eye movement periods

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(SOREMPs), but none of the polysomnographic variables was associated with subjective or objective measures of sleepiness. Variant rs2859998 in UBXN2B gene showed a strong association (P = 1.28E-07) with the age at onset of excessive daytime sleepiness, and rs12425451 near the transcription factor TEAD4 (P = 1.97E-07) with the age at onset of cataplexy. Altogether, our results indicate that the diagnostic delay remains extremely long, age and gender substantially affect symptoms, and that a genetic predisposition affects the age at onset of symptoms.

INTRODUCTION

Narcolepsy with cataplexy (NC) affects ~20 per 100 000 individuals, with an incidence of ~0.3–0.6 per 100 000 person-years (Longstreth *et al*., 2007; Partinen and Hublin, 2011; Poli *et al*., 2013). Evidence indicates that NC is caused by the loss of ~70 000 hypocretin cells in the hypothalamus, and the best biological marker for human narcolepsy is the reduction (or complete deficiency) of hypocretin-1 in the cerebrospinal fluid (CSF; Nishino *et al*., 2000). NC displays a strong genetic predisposition: more than 92% of Caucasian patients (Mignot *et al*., 2001) carry the human leukocytes antigen (HLA)–*DQB1*06:02*, while HLA–*DQB1*06:03* allele confers a strong protection (Hor *et al*., 2010). The tight association with specific HLA alleles, the recent discovery of circulating anti-TRIBBLES-2 antibodies (Cvetkovic-Lopes *et al*., 2010), the potentially elevated antistreptolysin O titers close to disease onset (Aran *et al*., 2009), the association with a polymorphism of T-cell receptor alpha gene and a polymorphism of the purinergic receptor *P2RY11* gene (Kornum *et al*., 2011) are strong indications of an autoimmune origin. However, the exact mechanism of such an autoimmune process remains unknown.

Despite major advances in understanding the pathophysiology of the disease and despite the fact that narcolepsy is a serious chronic sleep disorder with a major impact on performance, social relations, quality of life and socio-economic burden (Jennum *et al*., 2012), many cases remain undiagnosed (Dodel *et al*., 2007; Ozaki *et al*., 2008; Vignatelli *et al*., 2004). Variation in severity of the condition with incomplete or atypical forms and variable symptoms might contribute to under-diagnosis. Also, narcolepsy may be misdiagnosed as another sleep disorder, psychiatric disorder, epilepsy or side-effect of medication, or by lack of knowledge of the clinical characteristics (Morrish *et al*., 2004). In addition, the exact nosology of narcolepsy is still controversial for different reasons.

1. Narcolepsy with cataplexy can be diagnosed by history alone (American Academy of Sleep Medicine, 2005) but this approach may not be accurate (Morrison *et al*., 2011), as excessive daytime sleepiness (EDS) is a common feature of many sleep disorders, and cataplexy has common characteristics with experiences reported by healthy subjects (Anic-Labat *et al*., 1999; Overeem *et al*., 2011). Even if EDS in narcolepsy is different from that in other sleep disorders, a precise description is missing in the international criteria for NC. Nevertheless, significant differences exist between the cataplexy-like episodes and clear-cut cataplexy, such as the triggers and the muscles involved (Anic-Labat *et al*., 1999; Sturzenegger and Bassetti, 2004). Additional symptoms, such as hypnagogic or hypnopompic hallucinations (HH) and sleep paralysis (SP) are frequent in the general population (Ohayon *et al*., 1996, 1999; Sharpless and Barber, 2011).
2. Patients with NC may test negative in the proposed multiple sleep latency test (MSLT) requirements. On the other hand, patients with sleep apnea (Chervin and Aldrich, 2000) and even normal subjects (Mignot *et al*., 2006) may show short MSLT latencies and sleep-onset rapid eye movement periods (SOREMPs). Even if the prevalence of SOREMPs during MSLT in the general population has not been exhaustively studied, some evidence (Singh *et al*., 2006) suggests that 3.9% of the general population can be positive for multiple SOREMPs. Aldrich underlined that 30% of patients in an average sleep laboratory fulfilling both criteria, a mean sleep latency <5 min and two or more SOREMPs, do not have narcolepsy (Aldrich *et al*., 1997). With the more recent limit of <8 min this percentage will even be greater.
3. From a clinical point of view, the spectrum of NC is still far from being clearly delineated. The nosological limits between narcolepsy without cataplexy (presenting with all narcolepsy symptoms but cataplexy), with similar polysomnographic (PSG) and MSLT abnormalities (Dauvilliers *et al*., 2003; Mignot *et al*., 2002), idiopathic hypersomnia without long sleep time, and non-organic hypersomnia (Kaplan and Harvey, 2009) are in some cases imprecise. There are many common clinical aspects between narcolepsy without cataplexy and idiopathic hypersomnia without long sleep time (note also that the delay between EDS and cataplexy onset may be highly variable), which may point to the need of confirming proposed diagnostic criteria (Bassetti and Aldrich, 1997; Bassetti *et al*., 2003; Billiard, 2007).
4. Although NC is tightly associated with HLA, genotype can only support diagnosis, as the main susceptibility allele (*DQB1*06:02*) is also found in about 25% of healthy controls (Mignot *et al*., 2001).
5. Although NC is associated with low or undetectable levels of hypocretin-1 in the CSF (normal hypocretin levels in NC are exceptional; Knudsen *et al*., 2010), this

measure is not routinely used in clinical practice. In addition, low CSF hypocretin-1 levels were described in other neurological or sleep disorders, such as in certain cases of Guillain–Barré syndrome, anti-Ma encephalitis, late stage of Parkinson's disease, head trauma and in Prader–Willi syndrome (Baumann *et al.*, 2008; Fronczek *et al.*, 2009; Mignot *et al.*, 2002; Overeem *et al.*, 2004; Ripley *et al.*, 2001b).

6. Many studies emphasize the increased body mass index (BMI) in patients with narcolepsy, as compared with either idiopathic hypersomnia or the general population (Kok *et al.*, 2003; Poli *et al.*, 2009), but the relationship between the metabolic alterations and the duration of untreated disease was not explored.

Given the preceding limitations, the aim of the present study was to provide a reference detailed clinical and PSG description of a large sample of patients with NC and their eventual genetic predisposition. A major aim was to establish the relationship between symptoms and the diagnostic delay. To the best of our knowledge, our sample is the largest population of well-defined NC ever reported.

PATIENTS AND METHODS

Patients were diagnosed in affiliated sleep centers of the European Narcolepsy Network (EU-NN): France (n = 339); the Netherlands (n = 219); Germany (n = 185); Spain (n = 157); Italy (n = 68); Switzerland (n = 52); Denmark (n = 41); Poland (n = 30); and Slovakia (n = 8), with a final sample of 1099 patients. The data were gathered as a retrospective EU-NN database during a European genome-wide association study (GWAS; Hor *et al.*, 2010) comparing sporadic cases of narcolepsy with HLA-matched controls. Patients were examined by physicians experienced with narcolepsy, and the diagnosis was based on diagnostic criteria of the ICSD-2 (American Academy of Sleep Medicine, 2005; Billiard, 2007). All patients had narcolepsy with unambiguous clear-cut cataplexy, defined as sudden episodes of muscle weakness triggered by emotions. Given the differences between sporadic and familial cases (e.g. the frequency of HLA–*DQB1*06:02* negative and CSF hypocretin-1 normal cases; Mignot, 1998; Mignot *et al.*, 2002), suggesting a potential different pathogenesis (as demonstrated in the canine model of narcolepsy; Ripley *et al.*, 2001a), only sporadic cases were included. All patients were from European origin and were HLA–*DQB1*06:02* positive. The data were derived from a number of research projects, some published (e.g. Dauvilliers *et al.*, 2001; Hor *et al.*, 2010). Local ethics committees approved the recruitment of patients for research protocols, and all patients gave their consent to participate.

Patients were investigated in terms of the following.

1. Demographic characteristics: date of birth, gender, height, weight, BMI at diagnosis. Circumstances at onset (triggering factors) were not available in most patients.
2. Age at EDS onset and age at cataplexy onset. We defined the age at onset of NC as the age at occurrence of EDS and/or cataplexy, determined during the clinical interview.
3. Frequency of cataplexy attacks at diagnosis. The frequency of cataplexy was assessed by a scale from 1 to 5, reporting rare to very frequent cataplexy attacks (Dauvilliers *et al.*, 2001): 1 = one or less cataplexy attacks per year; 2 = more than one cataplexy attack per year but less than one per month; 3 = more than one attack per month but less than one per week; 4 = more than one per week but less than one per day; 5 = at least one cataplexy attack per day.
4. Epworth Sleepiness Scale (ESS) score at diagnosis.
5. Polysomnographic variables [including apnea–hypopnea index (AHI) and periodic leg movements during sleep index (PLMSI) when available] and MSLT results (mean sleep latency, number of SOREMPs) at diagnosis. Even if the recording procedures were different amongst centers, most of the patients underwent nocturnal in-lab PSG followed by an MSLT as part of the diagnostic evaluation. For PSG and MSLT, sleep latency was defined as the time from lights off to the first epoch scored as sleep. A SOREMP was defined as the occurrence of an epoch of REM sleep within 15 min after the first epoch scored as sleep. Although MSLT was performed according to standard methods (Carskadon *et al.*, 1986), the number of scheduled naps could be variable. To standardize the results, we calculated the percentage of SOREMPs of the total number of naps: percentage of naps with SOREMPs = [(number of SOREMPs/number of MSLT sessions) $\times$ 100].
6. Cerebrospinal fluid hypocretin-1 (measured by Phoenix RIA kit) level when available.
7. Human leukocytes antigen–*DQB1* genotyping was available from affiliated HLA or blood centers (at least 4 digits typing by standard techniques).
8. Associated features, with particular attention to symptoms frequently reported with narcolepsy: SP; HH; and poor nocturnal sleep.
9. Co-morbidities (sleep-related, somatic or psychiatric) and treatment when available.
10. Genome-wide association study: to test genetic associations with clinical traits; age and gender were used as covariates whenever they showed nominally significant association with the tested trait ($P < 0.05$). Continuous clinical phenotypes were inverse normal quantile transformed before applying linear regression [including relevant covariates and single nucleotide polymorphism (SNP) imputed allele dosage]. For dichotomous clinical traits logistic regression was used.

Statistical analysis

Descriptive analyses were performed for: gender; age at diagnosis; age of symptom onset; symptoms at diagnosis;

BMI, ESS, PSG and MSLT results; hypocretin-1 level; frequency of co-morbidities and symptomatic treatments. The interval between symptoms' onset and diagnosis was calculated. The gender effect on demographic (age at symptoms' onset, age at diagnosis, diagnostic delay, BMI) and clinical variables (PSG, MSLT, sleepiness, frequency of cataplexy and hypocretin level) was analysed using *t*-test/Kruskal–Wallis/χ^2 test. Additional statistical analyses were carried out using, when appropriate, *t*-test/ANOVA/Kruskal–Wallis to test the effect of origin, BMI, sleepiness and frequency of cataplexy. A possible interaction between BMI and gender was evaluated by two-way ANOVA. Because not all variables were available in all patients, the number of patients in each test was different. To verify that the outcome of our statistical tests was not affected by sub-sampling for each variable, the effects of age and gender on BMI, diagnostic delay and sleep characteristics were analysed by multivariate regression analysis in a reduced sample with complete information for variables of interest (n = 611). This analysis did not reveal any major difference as compared with analyses including the maximum number of patients for each variable. Correlation coefficient (Pearson or Spearman) was used to assess the relationship between demographic and clinical variables. Statistical significance for all tests was assumed when $P < 0.05$. For the entire sample and separately, for each gender, we have performed principal component analysis taking into account demographic, clinical, PSG, MSLT and laboratory data. All statistical tests were computed using PASW 18.0 Statistics (IBM SPSS Statistics, Armonk, NY, USA).

RESULTS

Demographic data

Data from 1099 patients were analysed. Table 1 shows demographic characteristics of our sample. The changes in sample size occurred because of missing data. Discrepancies or absence of information in the charts were labeled as data missing. For some data, manifestly outlier values were removed [e.g. total sleep time (TST) <2 h, MSLT latency >20 min, etc.].

In our sample, 54.8% of the patients were men and 45.2% women. The birth distribution ranged from 1905 to 2004. No predominance of any month or season was observed for birth. The mean age at diagnosis was 36.9 ± 17.1 years (range: 4–87 years). The age of diagnosis varied depending on the country of origin, with a mean age at diagnosis ranging from 33.1 ± 16.8 years in France to 43.9 ± 17.6 years in Spain (one-way ANOVA for factor 'origin', F = 9.234, $P < 0.001$), but it was similar in women (36.9 ± 16.9 years) and men (36.8 ± 17.4 years). Age at diagnosis and diagnostic delay were strongly correlated (r = 0.799, $P < 0.001$). The strength of this correlation was maintained even after excluding patients younger than 20 years old from the analysis (r = 0.714, $P < 0.001$).

In most of the patients both EDS and cataplexy appeared at the same time (48.8%), EDS preceded the onset of cataplexy in 43.8% and cataplexy was the first reported symptom in 7.4% of patients only. There were no significant differences between countries or by gender. Based on clinical interview, the age at EDS onset could be established in 990 patients. The mean age at EDS onset was 22.7 ± 11.9 years (range: 3–80 years; median: 20 years), with a significant gender difference (*t*-test; Mann–Whitney U = 107 302, P = 0.003; 23.7 ± 12.4 years in men versus 21.5 ± 11.8 years in women) but no difference between countries. The mean age at cataplexy onset (n = 685) was 25.8 ± 12.8 years (range: 5–80 years; median: 24 years), with a normal distribution in both genders. The age of cataplexy onset was significantly different between countries (F = 3.088, P = 0.009; minimum in Italy, 23.0 ± 12.1 years; maximum in Germany, 32.9 ± 11.3 years). In seven patients, age at symptoms' onset was older than 60 years (EDS was the first symptom or appeared at the same time as cataplexy). The mean delay between EDS onset and cataplexy onset (n = 678) was 2.8 ± 8.0 years. The maximum delay observed between EDS and cataplexy onset was

Table 1 Demographic characteristics of the sample

Parameter	n *(All)*	*Mean ± SD*	*Men*	*Women*	t/χ^2	P
Age at diagnosis (years)	755	36.87 ± 17.13	36.80 ± 17.40	36.91 ± 16.86	NS	NS
Age at EDS onset (years)	990	22.73 ± 11.88	23.74 ± 12.43	21.49 ± 11.84	107 302	0.003*
Age at cataplexy onset (years)	685	25.80 ± 12.84	26.05 ± 13.25	25.45 ± 12.25	NS	
Diagnostic delay	738	14.63 ± 14.31	13.82 ± 13.79	15.62 ± 14.94	60 950	0.044*
First symptom (%)					NS	
Cataplexy	47	7.40	8.10	6.50		
Cataplexy and EDS	310	48.80	51.30	45.50		
EDS	278	43.80	40.60	48.00		
BMI	903	27.34 ± 5.59	27.77 ± 4.56	26.80 ± 6.55	82 629	<0.001
Gender (% men)	590	54.80				

*Mann–Whitney U, *t*-test for ranks; *t*: *t*-test; χ^2: Fisher's exact test.
BMI, body mass index; EDS, excessive daytime sleepiness.

48 years, and the maximum delay between cataplexy onset and EDS was 40 years.

Available data from 738 patients revealed that the mean delay between the onset of the first clinical symptom (EDS or cataplexy) and diagnosis was 14.6 ± 14.3 years (median 10.5 years; range from <1 to 67 years). The delay between disease onset and diagnosis was larger for patients with later age at onset, and for those with EDS as first symptom ($r = 0.169$, $P < 0.001$). An important variability between countries was observed for diagnostic delay ($F = 8.042$, $P < 0.001$), with the lowest delay in France (11.9 ± 13.7 years) and the highest one in Spain (21.1 ± 14.7 years). Nevertheless, diagnostic delay was influenced by the time of diagnosis (for the analysis of the time of diagnosis, groups were created by 10-year intervals). For all countries, cases diagnosed before 1991 have a significantly decreased delay (mean diagnostic delay before 1991 was 8.0 ± 4.7 years, compared with the ones diagnosed after). One explanation could be the accumulation in time of undiagnosed cases. The higher number of cases diagnosed before 1991 was in France, while in Spain the increase in number of cases was observed starting with 1991. For the entire sample, the best predictors of diagnostic delay (forward stepwise regression) were age at diagnosis, first symptom and the frequency of cataplexy (Table 2).

Clinical and laboratory data

BMI

The mean BMI at diagnosis ($n = 903$) was 27.3 ± 5.6 kg m^{-2}. About two-thirds of the patients were overweight or obese (36.5% had a BMI between 25 and 29.9 kg m^{-2}, and 27.2% a BMI > 30 kg m^{-2}). In patients with a normal weight range (BMI < 25 kg m^{-2}) there was a predominance of women (men versus women: 41.8% versus 58.2%, $\chi^2 = 40.867$, $P < 0.001$), for overweight patients a predominance of men was observed (BMI between 25 and 29.9 kg m^{-2}, men versus women: 66.7% versus 33.3%; BMI > 30 kg m^{-2}, men versus women: 54.5% versus 45.5%; $\chi^2 = 21.023$, $P < 0.001$). Patients with a normal BMI had a lower age at diagnosis, age of first symptom onset and diagnostic delay. To control for gender by BMI interaction, the age at diagnosis, age at symptom onset and diagnostic delay were also analysed by two-way ANOVA. Results indicated that gender and BMI affected all three variables, but none was affected by gender by BMI interaction ($P > 0.3$ for all comparisons). We also observed significant differences in PSG data [TST, sleep efficiency (SE), slow-wave sleep (SWS) and REM sleep duration are reduced, and S1 duration is increased in overweight and obese patients most probably due to an increase in sleep apnea severity; Table 3]. Nevertheless, none of the variables that assess the severity of sleepiness (the mean sleep latency during MSLT, number of SOREMPs, ESS), or frequency of cataplexy and hypocretin-1 levels, was different between patients with normal BMI versus overweight/obese.

Table 2 Forward stepwise regression for best predictors of diagnostic delay

Step no.	*Vars. entered*	r	R^2	P
1	Age at diagnosis	0.714	0.509	0.006
2	First symptom	0.732	0.536	<0.001
3	Cataplexy frequency	0.738	0.545	<0.001

Subjective sleepiness

Subjective sleepiness was assessed using the ESS in 803 patients at the time of diagnosis. The mean ESS was 17.4 ± 3.9 with a median score at 18. Only 38 patients (4.7%) had an ESS in the 'normal ranges' (<11). None of the 'non-sleepy' patients was under stimulant medication at diagnosis. The mean age at diagnosis in this group was not different from that of the group with ESS score > 10 (31.6 ± 14.9 years versus 37.3 ± 17.4 years), but the diagnostic delay was shorter (7.8 ± 7.0 years versus 14.7 ± 14.6 years, $P = 0.011$; Table 4). The first symptom in 'non-sleepy' patients was cataplexy in 12%, higher than in those who declared themselves sleepy (7.2%), but the difference was not statistically significant. The variables correlated with sleepiness were different between these two groups of patients, but the severity of narcolepsy symptoms was similar. The number of SOREMPs was significantly higher in patients with high ESS scores (67.94% in high ESS versus 17.32% in normal ESS, $t = 15.33$, $P < 0.0001$).

Cataplexy

The frequency of cataplexy was assessed on a scale from 1 to 5, from rare to very frequent cataplexy attacks. In our case series ($n = 829$), almost two-thirds of the patients had frequent or very frequent cataplexy attacks (scores 4 + 5: 62.6%; score 5: 41.9%), and 48 patients (5.8%) had very rare cataplexy attacks. The mean frequency of cataplexy score was 3.7 ± 1.3, which corresponds to more than one attack per month. Patients with severe cataplexy (score 5) had a reduced diagnostic delay (11.9 ± 12.8 years versus 18.3 ± 15.8 years, $P < 0.001$), and the percentage of those who experienced sleepiness, HH and SP was higher in patients with severe cataplexy (Table 5).

Associated features

Hypnagogic or hypnopompic hallucinations were experienced by 63.1% of patients (370 patients), and 52.6% (257 patients) experienced SP; both symptoms were present in 43.6% (175 patients). Only 28.2% (303 patients) of the entire patient population reported either SP or HHs alone (Table 6).

Table 3 Demographic, clinical and PSG characteristics of the patients, according to BMI

Parameter	n	*BMI < 25 kg m^{-2}*	*BMI 25–29.9 kg m^{-2}*	*BMI > 30 kg m^{-2}*	*F/K–W*	P
Age at diagnosis (years)	699	30.54 ± 15.12	42.30 ± 17.41	39.57 ± 17.04	35.77	<0.001
Age at EDS onset (years)	878	19.94 ± 10.29	25.38 ± 12.85	23.62 ± 12.10	17.66	<0.001
Age at cataplexy onset (years)	642	22.78 ± 11.87	29.52 ± 13.76	25.74 ± 12.30	16.924	<0.001
Diagnostic delay	686	11.02 ± 12.07	16.70 ± 15.40	17.45 ± 15.58	14.547	<0.001
First symptom (%)	597					
Cataplexy		6.70	8.70	6.50	1.003	0.606
Cataplexy and EDS		47.30	46.30	53.50		
EDS		46.00	45.00	40.00		
Gender (% men)		41.80	66.70	54.50	3.536	<0.001
HH (% positive)		65.80	62.50	59.00	2.396	0.302
SP (% positive)		54.80	55.30	50.20	1.501	0.472
HH and SP (% positive)		45.70	45.00	41.30	2.519	0.281
EDS, HH and SP (% positive)		44.40	42.00	38.30	3.041	0.219
ESS score		17.16 ± 3.91	17.63 ± 3.83	17.58 ± 3.91	1.209	0.3
EDS (% positive)		95.10	95.60	95.00	0.094	0.954
Frequency of cataplexy (%)						
1 (one or less cataplexy attacks per year)		4.70	8.90	3.70	2.201	0.333
2 (more than one cataplexy attacks per year, but less than one per month)		13.40	11.70	14.30		
3 (more than one attack per month, but less than one attack per week)		18.10	20.60	16.40		
4 (more than one attack per week, but less than one per day)		22.00	17.50	19.00		
5 (at least one cataplexy attack per day)		41.90	41.20	46.60		
Sleep latency in PSG						
TST	686	427.22 ± 69.70	407.38 ± 82.69	392 ± 80.59	22.969	<0.001
SE	761	86.51 ± 16.99	82.59 ± 11.90	81.76 ± 12.04	35.857	<0.001
Stage1 (% of TST)	722	12.16 ± 8.76	15.26 ± 10.65	16.72 ± 10.72	27.248	<0.001
Stage 2 (% of TST)	721	43.56 ± 11.27	44.26 ± 12.78	42.82 ± 12.56	1.601	0.449
SWS (% of TST)	753	19.50 ± 9.53	15.97 ± 10.00	17.20 ± 8.53	21.792	<0.001
REM (% of TST)	733	20.26 ± 7.51	19.41 ± 8.34	17.21 ± 8.53	14.281	0.001
REM SOL	743	47.03 ± 55.51	59.24 ± 64.23	52.42 ± 65.50	2.316	0.314
SOL	752	9.97 ± 15.20	10.57 ± 30.82	8.61 ± 13.61	2.906	0.234
Mean sleep latency at MSLT	816	3.98 ± 3.06	3.78 ± 3.06	3.79 ± 2.70	1.164	0.559
Number of SOREMP (%)	777					
0–15		4.40	4.40	3.90	0.382	0.826
20–40		21.10	21.60	18.60		
50–75		29.60	32.40	36.10		
80–100		44.90	41.80	41.40		
Hypocretin (%)						
0–40 pg mL^{-1}		80.20	89.80	90.10	5.186	0.75
>40 pg mL^{-1}		19.80	10.20	9.90		

F-value: derived from one-way ANOVA.
BMI, body mass index; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HH, hypnagogic or hypnopompic hallucination; K–W, Kruskal–Wallis test for not normally distributed variables; MSLT, multiple sleep latency test; PSG, polysomnography; REM, rapid eye movement; SE, sleep efficiency; SOL, sleep-onset latency; SOREMP, sleep-onset REM period; SP, sleep paralysis; SWS, slow-wave sleep; TST, total sleep time.

PSG features

Table 7 shows PSG and MSLT characteristics of patients with NC. The mean TST was 411.7 ± 80.4 min, with a mean SE of 83.8 ± 11.5%; 42.8% had abnormal SE < 85%. The age at diagnosis was negatively correlated with TST ($r = -0.389$, $P < 0.001$), SE ($r = -0.335$, $P < 0.001$), SWS duration ($r = -0.293$, $P < 0.001$) and REM sleep duration ($r = -0.209$, $P < 0.001$), and positively with S1 duration ($r = 0.315$, $P < 0.001$). The mean number of stage shifts was 108.3 ± 64.6, and the mean wake after sleep onset was 59.5 ± 43 min. The mean sleep-onset latency (SOL) was

Table 4 Significant differences due to sleepiness (EDS: upper, MSLT: lower table)

Variable	n	*ESS 0–10*	*ESS > 10*	t	P
Diagnostic delay	627	7.81 ± 7.04	14.7 ± 14.6	6.488	0.011
SE	691	87.06 ± 11.2	83.47 ± 11.17	5.491	0.019
Mean sleep latency on MSLT	774	5.99 ± 3.94	3.75 ± 2.9	15.307	<0.001
Number of SOREMP (%)	803				
0–15		84.20	0.10	90.849	<0.001
20–40		10.50	21.50		
50–75		NA	34.50		
80–100		5.30	43.80		
Variable	*n*	MSLT ≤ 8	MSLT > 8	χ^2/t	*P*
Age at EDS onset (years)	870	23.36 ± 12.14	19.8 ± 11.03	6.727	0.009
Age at cataplexy onset (years)	623	26.33 ± 12.93	21.94 ± 11.55	5.305	0.021
ESS	774	17.73 ± 3.69	14.95 ± 4.26	30.978	<0.001
Number of SOREMP (%)	788				
0–15		3.00	9.80	21.293	<0.001
20–40		18.80	40.90		
50–75		33.60	22.90		
80–100		44.70	26.20		

EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; MSLT, multiple sleep latency test; SE, sleep efficiency; SOREMP, sleep-onset rapid eye movement period.

Table 5 Significant differences due to the severity of cataplexy

Variable/frequency of cataplexy	*1*	*2*	*3*	*4*	*5*	*F/K–W*	P
Diagnostic delay	16.67 ± 14.76	18.30 ± 15.84	15.11 ± 14.29	14.88 ± 14.59	11.91 ± 12.82	4.480	0.001
EDS, HH and SP (% positive)	17.50	28.70	42.50	40.60	51.40	10.125	<0.001

F-value: derived from one-way ANOVA.
EDS, excessive daytime sleepiness; HH, hypnagogic or hypnopompic hallucination; K–W, Kruskal–Wallis test for not normally distributed variables; SP, sleep paralysis.

Table 6 Clinical variables

Parameter	*n (all)*	*Mean ± SD*	*Men*	*Women*	t-*test*	P
HH (% positive)	587	63.10	58.90	68.00	8.030	0.005
SP (% positive)	488	52.60	50.20	55.50	NS	
HH and SP (% positive)	402	43.60	40.40	47.60	6.622	0.01
EDS, HH and SP (% positive)	301	41.80	38.90	44.60	NS	
ESS score	803	17.45 ± 3.86	17.33 ± 3.91	17.57 ± 3.80	NS	
EDS (% positive)	765	95.30	95.40	95.10	4.488	0.034
Frequency of cataplexy (%)						
1 (one or less cataplexy attacks per year)	48	5.80	6.80	4.60	NS	
2 (more than one cataplexy attacks per year, but less than one per month)	108	13.00	11.40	15.30		
3 (more than one attacks per month, but less than one attack per week)	154	18.60	18.60	18.50		
4 (more than one attack per week, but less than one per day)	172	20.70	19.20	22.60		
5 (at least one cataplexy attack per day)	347	41.90	44.10	39.00		

EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HH, hypnagogic or hypnopompic hallucination; SP, sleep paralysis.

Table 7 PSG and MSLT variables and hypocretin-1 levels

Parameter	*n (all)*	*All mean ± SD*	*Men*	*Women*	t-*test*	P
TST	751	411.70 ± 80.45	410.33 ± 79.85	414.26 ± 80.38	NS	
SE	848	83.80 ± 11.53	82.86 ± 11.20	84.95 ± 11.82	74 791	<0.001*
Stage 1 (% of TST)	772	14.62 ± 10.36	16.46 ± 10.89	12.96 ± 9.21	55949.9	<0.001*
Stage 2 (% of TST)	773	43.75 ± 12.3	43.04 ± 12.55	44.74 ± 11.91	66203.5	0.03*
SWS (% of TST)	808	17.54 ±9.97	16.36 ± 9.88	18.86 ± 9.89	67725.5	<0.001*
REM (% of TST)	788	19.17± 8.54	19.27 ± 8.16	19.04 ± 8.98	NS	
REM-onset latency	835	54.59 ± 65.30	55.03 ± 67.39	53.92 ± 62.91	NS	
SOL (PSG)	844	10.33 ± 23.80	11.86 ± 28.35	9.30 ± 14.75	NS	
Mean sleep latency (MSLT)	927	3.92 ± 3.03	4.10 ± 3.23	3.67 ± 2.70	NS	
Number of SOREMP (%)						
0–15	34	4.00	3.80	4.30	NS	
20–40	169	20.50	22.90	17.70		
50–75	265	32.10	27.90	36.80		
80–100	358	43.40	45.30	41.30		
Hypocretin (%)						
Undetectable (<40 pg mL^{-1})	254	86.40	55.11	44.89		
(>40 pg mL^{-1})	40	13.60	57.50	42.50		

*Mann–Whitney *U*-test, *t*-test for ranks.
MSLT, multiple sleep latency test; PSG, polysomnography; REM rapid eye movement; SE, sleep efficiency; SOL, sleep-onset latency; SOREMP, sleep-onset REM period; SWS, slow-wave sleep; TST, total sleep time.

10.3 ± 23.8 min, with a mean REM sleep latency (REM SL) of 54.6 ± 65.30 min. Thirty-five percent of patients had a SOREMP during the night PSG (REM SL < 15 min), and only 14.2% had a REM SL between 90 and 150 min, with some patients showing prolonged REM latency (REM SL > 150 min; 9.1%). The distribution of REM SL was bimodal, with a first peak between 0 and 10 min after sleep onset, and a second less marked peak at 50–70 min. Women had a better SE, longer S2 and SWS duration, and shorter S1.

To evaluate whether the ESS and/or MSLT results are influenced by night sleep, a multiple regression analysis was performed, controlling for age at diagnosis and gender. None of the variables was retained in the analysis, suggesting that changes in sleep structure or duration are due to age and gender, but not to the condition.

The mean sleep latency during MSLT in 927 patients (without treatment) was 3.9 ± 3.0 min, with a median at 3 min, indicating severe sleepiness; 92% of the patients had a sleep latency < 8 min (Table 7). Although small, the mean sleep latency at MSLT was significantly shorter in women (3.68 ± 2.7 min versus 4.10 ± 3.24 min in men). The mean percentage of SOREMPs during the MSLT was 65.9 ± 27.2%, which corresponds roughly to 3 SOREMPs during a 5-naps MSLT. However, 3.9% of the sample had no SOREMPs during the MSLT, and an additional 5.7% had 20% SOREMPs, which corresponds to 1 SOREMP during a 5-naps MSLT. Laboratory criteria currently used to diagnose narcolepsy (sleep latency shorter than 8 min and at least 2 SOREMPs) were present in 90.3% of patients. We found a negative correlation between the mean sleep latency at the MSLT and the number of SOREMPs ($r = -0.326$, $P < 0.001$). Reduced MSLT mean sleep latency predicted SOREMPs ($r = -0.326$, $P < 0.001$). The MSLT latency was negatively correlated with the ESS ($r = -0.306$, $P < 0.001$).

CSF hypocretin-1

Cerebrospinal fluid hypocretin-1 measurements were available in 294 patients (Table 7). In 96.3% of the patients hypocretin-1 levels were undetectable (<40 pg mL^{-1}, $n = 252$) or <110 pg mL^{-1} ($n = 31$). In the remaining 11 patients, intermediate (four cases) to normal levels (seven cases) were obtained (from 118 to 400 pg mL^{-1}). There were no significant correlations between hypocretin-1 levels (analysed as bivariate parameter: <40 pg/ml and more than 40 pg/ml) and variables of interest; there were no differences in clinical features between patients with undetectable and those with hypocretin-1 levels between 40 and 110 pg mL^{-1}. Regarding the 11 patients with intermediate to normal values of hypocretin-1, the only significant difference was observed in the frequency of cataplexy, which was lower (frequency code 1–2: 42.9% versus 11% in patients with low/undetectable levels of hypocretin-1, $P = 0.004$).

Effect of age and gender on NC features and diagnostic delay

Diagnosis is more delayed in women of all age groups (two-way ANOVA for 'age group' and 'gender', $P < 0.001$; no interaction; men versus women: 13.8 ± 13.8 years versus 15.6 ± 14.9 years), independently from the origin or the time at diagnosis. Stage 1 was increased in men in all age groups (men versus women 16.5 ± 10.9% versus 13.0 ± 9.2%; Mann–Whitney *U*: 55949, $P < 0.001$). Younger men were sleepier than younger women (ESS in men 20–30 years old

versus women 17.4 ± 3.8 versus 16.8 ± 3.9, $P = 0.04$), but with aging women declared themselves sleepier (ESS in men >60 years old versus women 17.6 ± 3.8 versus 18.8 ± 3.8, $P = 0.034$). The same pattern was observed in parameters correlated with sleepiness: mean SOL during MSLT (young men versus young women 3.7 ± 2.7 min versus 4.04 ± 2.6 min, $P = 0.04$; men > 60 years old versus women 4.1 ± 3.6 versus 3.9 ± 2.8, $P = 0.53$) and SOREMPs (young men versus young women 67.3 ± 28.1 versus 63 ± 29; men > 60 years old versus women 59.0 ± 26.2 versus 70.8 ± 25.0, $P = 0.014$).

Principal component analysis

For variables relevant from a clinical point of view we performed a principal component analysis, varimax rotation with Kaiser normalization (KMO measure of sample adequacy 0.611; Bartlett's test of sphericity: chi square 1010.20; $P < 0.001$). This analysis was performed for the entire sample and, separately, for each gender. Table 8 presents the major extracted components (eigenvalue > 1; no restriction on selected number of components). For the entire sample, eight components accounted for 67.05% of phenotype variability. By gender analysis retrieved only seven components for each gender.

For the entire sample, the first major component (% variance explained = 14.33%) included age- and PSG-related variables: age at diagnosis; diagnostic delay and non-REM sleep stage 1 (positively); TST and SE (negatively; Table 8). The second component (% variance explained = 12.68%) accounted for sleepiness, with ESS score, presence of EDS, and number of SOREMPs during MSLT (positively) and mean MSLT (negatively). Components three and four (% variance explained = 7.7% and 7.6%, respectively) were represented by sleep variables. The frequency of cataplexy was extracted (positively) together with the presence of sleepiness, HHs and SP as part of component five (% variance explained = 7.1%). First symptom, diagnostic delay and BMI, SOL, and CSF hypocretin-1 levels with stage 1 (positively) and mean MSLT (negatively) were extracted separately within components 6–8. By gender analysis revealed effects that could not be assessed by other analysis. Compared with the entire sample, the most important variability in men was due to sleepiness and its correlates. In men, diagnostic delay was influenced by the severity of cataplexy and the first symptom (if cataplexy was the first symptom, the diagnostic delay was shorter). In women, those who had cataplexy as the first symptom had a lower REM-onset latency.

Other sleep-related disorders

Respiratory parameters were not systematically assessed in most sleep laboratories when evaluating a patient suspected

Table 8 Principal component analysis

Rotated component matrix

	Component							
	1	2	3	4	5	6	7	8
BMI	0.312	0.073	0.306	0.039	−0.191	−0.314	−0.210	0.023
Age at diagnostic	0.776	0.109	0.089	0.150	−0.195	0.157	−0.142	−0.074
Diagnostic delay	0.730	0.112	0.102	0.058	−0.198	0.331	−0.134	−0.199
Frequency of cataplexy	−0.027	−0.039	0.069	−0.053	0.733	−0.158	0.008	0.062
TST	−0.749	0.015	−0.076	0.086	−0.148	0.145	−0.153	−0.022
SE	−0.737	0.011	−0.048	0.012	−0.124	0.183	−0.189	−0.028
Stage 1	0.442	−0.045	0.460	−0.203	−0.014	−0.174	−0.248	0.429
Stage 2	−0.178	0.057	0.103	0.869	−0.095	0.124	0.120	−0.226
SWS	−0.279	0.093	−0.056	−0.754	−0.062	0.136	0.137	−0.290
REM	−0.165	0.038	−0.733	−0.178	−0.032	0.040	0.047	−0.028
SOL	0.089	0.008	0.085	0.007	−0.041	−0.047	0.875	0.094
REM latency	−0.002	0.011	0.731	−0.031	0.051	0.184	0.246	−0.173
Mean MSLT	0.061	−0.506	−0.031	0.039	0.193	0.120	−0.043	−0.330
No. SOREMPs	−0.027	0.871	0.026	0.007	0.037	0.014	−0.031	0.057
ESS score	0.098	0.885	−0.049	−0.029	0.049	0.073	−0.054	0.021
EDS	0.107	0.693	−0.025	0.031	0.245	0.041	0.069	−0.094
Hypocretin	−0.138	0.116	−0.125	0.034	0.090	0.228	0.135	0.738
EDS + HH + SP	0.002	0.261	−0.024	0.026	0.730	0.150	−0.044	−0.013
First symptom	0.005	0.038	0.080	0.010	−0.047	0.811	−0.057	0.137

BMI, body mass index; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HH, hypnagogic or hypnopompic hallucination; MSLT, multiple sleep latency test; REM, rapid eye movement; SE, sleep efficiency; SOL, sleep-onset latency; SOREMP, sleep-onset REM period; SP, sleep paralysis; SWS, slow-wave sleep; TST, total sleep time.

of NC. In 240 patients detailed breathing parameters were available. The mean AHI was 5.1 ± 10.6 per hour, with 26.3% having an AHI ≤ 5 per hour, 8.7% an AHI between 5 and 15 per hour, and 4.1% an AHI ≥15 per hour. The AHI was higher in men than in women (7.63 ± 13.3 versus 3.3 ± 6.7, $P = 0.007$), and correlated with BMI ($r = 0.208$, $P = 0.002$). No significant correlation was found with sleepiness (MSLT latency: $r = 0.012$, $P = 0.86$; ESS: $r = 0.36$, $P = 0.617$). Patients with AHI > 30 per hour tended to be older than those with AHI <30 per hour (40.5 ± 23 versus 35.3 ± 17.2, $P = 0.22$), and had higher BMI (30.6 ± 5.6 versus 27 ± 5.4, $P = 0.043$).

The PLMSI was available in 174 patients only. The mean PLMSI was 1.5 ± 25.8 per hour of sleep, with a median at 4.6 per hour; 49.4% had a PLMI > 5 per hour and 30.4% a PLMI > 15 per hour.

In a subgroup of 295 patients, the presence of REM sleep behavior disorder (RBD; confirmed during diagnostic PSG or clinically suspected) was established in 46% of men and 54% of women. No differences between patients with NC with and without RBD were found for sex distribution, age at diagnosis, ESS score or mean latency during MSLT.

Two-hundred and seventy patients were evaluated for other parasomnias than RBD. The most frequent parasomnias were: sleep talking (17%); arousal disorders (confusional arousals, sleepwalking, sleep terrors, in 8.1%); and bruxism (5.9%). Four patients (1.4%) exhibited characteristics of sleep-related eating disorders: one associated with arousal disorders, another associated with sleep talking, and two without other associated parasomnias.

Genome-wide association

To verify if any gene variant may be associated with narcolepsy phenotypes, we performed a GWAS in 585 patients who had been genotyped as part of a case–control GWAS recently published (Hor *et al.*, 2010). Among potential associations, 13 top hits were genotyped in 387 additional narcolepsy patients for replication. Table 9 shows the associations between the top 13 hits for the original, the replication and the whole sample metanalysed. None of the selected variants reached a genome-wide significance level ($P < 5E-08$). Nevertheless, a SNP (*rs2859998*) within the *UBXN2B*, an adaptor protein required for Golgi and endoplasmic reticulum biogenesis, was strongly associated (metanalysis $P = 1.28E-07$) with the age at onset of EDS. A similar strong association was also found between *rs12425451* near the transcription factor *TEAD4* and the age at onset of cataplexy (metanalysis $P = 1.97E-07$). The later association was the only significant one in the replication sample ($P = 0.048$), suggesting a potential true signal that needs further replication in other independent samples.

DISCUSSION

In this study we were able to analyse phenotypic and genetic data of 1099 sporadic HLA–*DQB1*06:02* positive patients with NC from the retrospective database of the EU-NN connecting sleep laboratories from France, the Netherlands, Germany, Spain, Italy, Denmark, Switzerland, Poland, Slo-

Table 9 Genome-wide association analysis of narcolepsy phenotypes

Phenotype	*rs#*	*aA*	*aB*	*x1*	*p1*	*x2*	*p2*	*x_meta*	*p_meta*
ESS (n1 = 418, n2 = 252)	rs16966122	A	G	−2.3655	9.11E-07	−0.8656	6.82E-02	−1.601	2.08E-06
Age EDS onset (n1 = 530, n2 = 319)	rs2859998	A	G	−4.6048	2.33E-07	−3.0064	2.20E-01	−4.4145	1.28E-07
Age EDS onset (n1 = 530, n2 = 319)	rs6072697	C	T	14.8434	4.29E-06	4.4731	2.76E-01	10.8306	1.84E-05
Age cataplexy onset (n1 = 435, n2 = 305)	rs12425451	C	T	−7.9529	7.30E-07	−4.5204	4.83E-02	−6.7866	1.97E-07
HH (n1 = 458, n2 = 310)	rs10160605	A	G	0.8865	3.84E-06	0.0294	8.48E-01	0.363	2.43E-03
TST (n1 = 442, n2 = 180)	rs304468	C	T	−28.7108	1.18E-06	9.1773	2.65E-01	15.7748	1.00E-03
TST (n1 = 442, n2 = 180)	rs890227	A	G	149.2937	4.11E-07	−16.656	3.77E-01	31.3251	4.82E-02
SE (n1 = 451, n2 = 268)	rs1515773	C	T	−5.6438	3.37E-07	3.083	1.80E-01	−3.9944	6.10E-05
SE (n1 = 451, n2 = 268)	rs2426087	A	G	11.638	4.30E-07	−1.9517	7.94E-01	10.463	1.98E-06
SOREMPs (n1 = 490, n2 = 298)	rs9397716	C	T	−11.7672	2.13E-05	0.0022	9.40E-01	0.0009	9.75E-01
SOREMPs (n1 = 490, n2 = 298)	rs9551427	A	G	−10.883	2.28E-05	0.0345	1.32E-01	0.0336	1.41E-01
BMI (n1 = 463, n2 = 307)	rs1882687	A	C	2.1251	3.90E-07	−0.3574	4.95E-01	1.1544	4.13E-04
REMS (n1 = 429, n2 = 260)	rs17294110	A	G	3.0476	8.91E-06	−2.5829	8.55E-02	2.0692	9.07E-04

rs#: SNP; aA and aB: minor and major alleles; x1 and x2: effect size for discovery and replication; meta: metanalysis; n1 and n2: number of patients in discovery and replication analyses.
BMI, body mass index; EDS, excessive daytime sleepiness; ESS, Epworth Sleepiness Scale; HH, hypnagogic or hypnopompic hallucination; REMS rapid eye movement sleep; SE, sleep efficiency; SOREMP, sleep-onset REM period; TST, total sleep time.

vakia, Czech Republic, Austria, Finland and UK. This is, to our knowledge, the largest published population of well-defined NC. We used this large sample of patients to further describe the clinical picture and the PSG characteristics of NC, to review the major features of NC, and genetic association with all available phenotypes. Below we will discuss the major findings.

Diagnostic phenotypes

Although NC can be clinically diagnosed by the presence of EDS and cataplexy, this is still not accurate enough in many cases, in particular in incomplete and atypical forms, or in patients with co-morbidities such as sleep apnea. In addition, EDS is common in other sleep disorders, and cataplexy, especially incomplete attacks, is not always easy to identify. For these reasons several other phenotypic criteria are widely used to support the diagnosis. Among these, HLA, hypocretin-1 and MSLT criteria are the most widely accepted ones. In our population, all cases had documented cataplexy and were HLA–*DQB1*06:02* positive, allowing us to evaluate the contribution of other phenotypic variables. Although available in only 294 patients (but still the largest sample ever reported), normal hypocretin-1 levels were found in only seven patients (2.38%), indicating, as reported by others, that hypocretin deficiency is the most reliable and sensitive biological marker for NC, even if not practically performed in everyday clinical practice. Among 927 patients with MSLT, 74 (8%) had a mean MSLT > 8 min and 79 (8.5%) had <2 SOREMPs, suggesting that MSLT criteria have a lower diagnostic value. Using the criteria proposed by Poli *et al.* (2013) (EDS and unambiguous cataplexy and MSLT mean sleep latency $\leq$8 min or MSLT SOREMPs $\geq$2), 96.9% of the patients met the criteria. Nevertheless, among 79 with < 2 SOREMPs, 41 (53%) had a SOREMP during the preceding night PSG. Adding the PSG SOREMPs to those with 1 SOREMP during MSLT brings the percentage of patients with SOREMPs > 2 up to 94.1%, suggesting a good diagnostic value that can be routinely used, as recently suggested (Andlauer *et al.*, 2012). Interestingly, among seven hypocretin-1 normal patients, MSLT data were available in three, and all three had mean MSLT <8 min with more than 2 SOREMPs. The same observation was found in two out of the four patients with intermediate hypocretin-1 levels. Overall, EDS, cataplexy and *DQB1*06:02* best characterize the condition, in the absence of hypocretin-1 measure. MSLT data together with PSG SOREMPs remain highly valuable, especially if the HLA typing is not available. Also note that in our population mean MSLT negatively correlated with the ESS score ($r = -0.302$, $P < 0.001$). Accordingly, principal component analysis identified ESS, mean MSLT and number of SOREMPs contributing to the same factor with strong correlations between them. In other disorders with EDS as the leading symptom this correlation cannot be demonstrated (Chervin and Aldrich, 1999; Shpirer *et al.*, 2006).

Time between narcolepsy onset and diagnosis

The delay between the first symptom and diagnosis has been previously reported to range from the same year to more than 60 years (Morrish *et al.*, 2004; Parkes *et al.*, 1995), usually more than a decade, with a mean delay between 15 and 17 years after the onset of EDS (Moldofsky *et al.*, 2000; Morrish *et al.*, 2004). Although it is generally claimed that the diagnostic delay has considerably shortened in recent years, in our population the mean diagnostic delay is still substantial (14.6 $\pm$ 14.3 years). Interestingly, patients with older age at onset had longer diagnostic delay, while younger patients (mainly children and adolescents) have the shortest diagnostic delay. Also, patients with EDS as the first symptom have longer diagnostic delay as compared with those with both EDS and cataplexy or cataplexy as the first symptom. Age at symptom onset and age at diagnosis varied amongst countries. Obviously several factors might be involved, including but not limited to differences in the recruitment population (child versus adult patients) and history of narcolepsy research in each country. Another interesting finding is the significant between-country differences in diagnostic delay, with the shortest in France (11.9 $\pm$ 13.7 years) and the longest in Spain (21.1 $\pm$ 14.7 years). Nevertheless, the diagnostic delay also depended on the year of diagnosis, with those diagnosed before 1991 having the shortest delay (8.0 $\pm$ 4.7 years). This paradoxical finding can be explained by a sharp increase in awareness and the number of narcolepsy specialists in the 1980s. In summary, the best predictors of a short diagnostic delay are the young age at diagnosis, the first symptom including cataplexy, and the higher frequency of cataplexy. These observations are in accordance with previous reports indicating that the year of symptom onset and whether or not cataplexy is one of the initial symptoms show a significant correlation with the diagnosis delay (Morrish *et al.*, 2004). Overall, the onset or worsening of cataplexy often prompts patients to look for medical assistance (Rye *et al.*, 1998), the interval between symptom onset and diagnosis is greater in patients whose symptom onset was further in the past (Dauvilliers *et al.*, 1998), and a reduction in the diagnosis delay is found in patients with a more recent date of birth (Furuta *et al.*, 2001).

Age and gender contributions

Gender differences in narcolepsy phenotypes are poorly investigated. Here we found a significantly longer diagnostic delay in women. Also, the age at onset for EDS was lower in women. These differences seem robust as there were no gender differences between countries or the time of diagnosis, while the age at onset for cataplexy was highly significantly different between countries. Mean sleep latency at MSLT was significantly shorter in women, in addition to several PSG and BMI differences. Our findings clearly indicate that narcolepsy symptoms are strongly affected by age and especially gender, needing further investigations.

Multivariate analysis

Using the most typical phenotypes in our multivariate analysis, we identified up to eight factors explaining their variance in a large NC population. As expected, the most significant one included age at diagnosis and diagnostic delay negatively correlated with TST and SE, and positively with the amount of stage 1 non-REM sleep. The second most significant component includes variables related to EDS, with ESS score and number of SOREMPs during MSLT being negatively correlated with mean sleep latency during MSLT. BMI segregated positively with stage 1 non-REM sleep and REM latency at night, and negatively with diagnostic delay and nocturnal REM sleep. Of interest, the levels of hypocretin-1 positively correlated with stage 1 non-REM sleep and negatively with mean latency during MSLT. Note that none of the night PSG variables was associated with subjective (ESS) or objective measures of sleepiness (MSLT), suggesting that EDS in narcolepsy does not result from poor quality night sleep, as already proposed (Broughton *et al.*, 1994). Overall, our analysis successfully identified groups of correlated symptoms, each explaining between 5.8 and 14.33% of phenotypic variance (total variance explained by eight factors = 67%).

Genetics of narcolepsy phenotype

We performed the first GWAS on narcolepsy clinical and laboratory phenotypes. Although many suggestive variants were found, only one, associated with the age at onset of cataplexy, showed a nominal significant association in the replication sample. The major explanation for this negative result is the complexity of the analysed phenotypes where genetic variants explain a small proportion of the phenotypic variance. Also, our original sample (585 patients) is somehow too small to have enough power to detect a genome-wide significant signal (note also that data for different phenotypes were not available in all 585 patients further reducing the statistical power). Nevertheless, the variant *rs12425451* associated with the age at onset of cataplexy is in the vicinity of the transcription factor TEAD4. TEAD4 is an important transcription factor controlling neuronal fate and survival (Cao *et al.*, 2008). This interesting finding needs replication.

CONCLUSION

In this large sample of well-defined patients with NC, our findings are compatible with previously published studies, extending data collected in smaller case-series. In addition we have identified several unexplored relationships between narcolepsy phenotypes. The long delay between the onset of symptoms and diagnosis implies that many cases still remain undiagnosed, and that major efforts need to be made to spread the knowledge of the disease among the population and in particular among physicians. Note that longer diagnostic delay is found here associated with higher BMI, suggesting that late diagnosis and/or undiagnosed cases are at higher risk for metabolic and cardiovascular diseases. Our findings also emphasize a large heterogeneity in the clinical presentation and laboratory variables of the disease, and point out the fact that accurate diagnosis needs to take into account the full clinical presentation and a critical interpretation of PSG and MSLT results. Although not routinely proposed, the measurement of hypocretin-1 concentration in the CSF could be an important diagnostic tool in dubious cases. Future studies should also take into account the gender as a major contributor.

Finally, we acknowledge several limitations of our study. Although the diagnosis was made in reference sleep laboratories with long-lasting expertise in narcolepsy following virtually identical diagnostic criteria, several procedures vary between laboratories, and complete and uniform data could not be obtained from all patients included, introducing some diagnostic site effects (a structured prospective database has been launched by the EU-NN in 2010). Some of the parameters analysed in the study (e.g. onset of EDS) were based on the reliance on subjects of correctly recalling symptoms, or in the analysis of retrospective data obtained from medical charts, which may vary according to differences in interpretation or in conducting clinical interviews between sites. We did not compare our patients with a reference population. Comparisons with subjects recruited from the same populations and diagnosed either with other hypersomnias or without any sleep disorder could have allowed identification of the most specific (in terms of specificity and sensitivity) narcolepsy phenotypes and comparisons of co-morbidities or the search for environmental risks factors implicated in the pathophysiology of the disease.

In conclusion, this study provides a detailed description of the clinical and PSG characteristics of a large and homogeneous group of patients with NC. Despite major advances during recent years in our understanding of the neurobiological basis of narcolepsy, NC is still an under-recognized condition. A better knowledge of the nosology of NC may allow earlier diagnosis of this life-long disabling condition.

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